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Comparative Study of the Conformational Lock, Dissociative Thermal Inactivation and Stability of *Euphorbia* Latex and Lentil Seedling Amine Oxidases

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The thermal stability of copper/quinone containing amine oxidases from *Euphorbia characias* latex (ELAO) and lentil seedlings (LSAO) was measured in 100 m*M* potassium phosphate buffer (pH 7.0) following changes in absorbance at 292 nm. ELAO was shown to be about 10°C more stable than LSAO. The dissociative thermal inactivation of ELAO was studied using putrescine as substrate at different temperatures in the range 47–70°C, and a "conformational lock" was developed using the theory pertaining to oligomeric enzyme. Moreover ELAO was shown to be more stable towards denaturants than LSAO, as confirmed by dodecyl trimethylammonium bromide denaturation curves. A comparison of the numbers of contact sites in inter-subunits of ELAO relative to LSAO led us to conclude that the higher stability of ELAO to temperature and towards denaturants was due to the presence of larger number of contact sites in the conformational lock of the enzyme. This study also gives a putative common mechanism for thermal inactivation of amine oxidases and explains the importance of C-terminal conserved amino acids residues in this class of enzymes.

KEY WORDS: Amine oxidase; conformational lock; *Euphorbia* latex; lentil; thermal inactivation; thermal stability.

1. INTRODUCTION

Copper/quinone-containing amine oxidases (amine: oxygen oxidoreductase deaminating; EC 1.4.3.6) catalyze the oxidation of primary amines with the formation of the corresponding aldehyde, ammonia and hydrogen peroxide. The oxidative deamination occurs by subtraction of two electrons from amines and transferring them to molecular oxygen by a ping–pong catalytic mechanism. All known AOs are homodimers in which each subunit (molecular mass approximately 70–90 kDa) contains one Cu ion and one tyrosine-derived 6-hydroxydopaquinone (TPQ)[6] as prosthetic groups (Janes *et al.*, 1990). These enzymes appear to be widespread and have been detected in bacteria (Cooper *et al.*, 1982), fungi and yeast (Corpillo *et al.*, 2003), plants (Medda *et al.*, 1995) and mammals (Medda *et al.*, 2004).

Di- and polyamines, such as putrescine, spermidine and spermine, play important role in cellular processes, e.g., cellular proliferation and tissue

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[6]Abbreviations: AO, amine oxidase; DTAB, dodecyl trimethylammonium bromide; ELAO, *Euphorbia* latex amine oxidase; LSAO, lentil seedling amine oxidase; PSAO, pea seedling amine oxidase; TPQ, 6-hydroxydopaquinone; T_{opt}, optimum temperature.